HPP-CFU. In summary, the higher concentration cytokine combination with IL-6 yielded more CD34+ cells and more CD34+CD38-DR-cells in 14 days culture. CD34+ cells expanded in the presence of lower concentration cytokine combinations present more differentiation character. IL-11 in either concentration resulted in a decrease in the progenitor cell numbers. At present, the most promising cytokine combination appears to be those containing IL-6 with high concentration cytokine combination. These findings help delineate the culture conditions that will support the expansion of cord blood for adult transplantation.

219

A NON-VIRAL METHOD OF TRANSFECTING MRNA FROM A NEUROBLASTOMA (NBL) CELL LINE (NGP) INTO IMMATURE DENDRITIC CELLS (DC)

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Background: DC are potent antigen presenting cells. mRNA from a NGP cell line, combined with a cationic lipid, is a non-viral method for transfecting immature DC. **Method:** To generate DC, Peripheral Blood Mononuclear Cells (PBMC) were isolated using Ficoll-Hypaque (Amersham Pharmacia) density gradient centrifugation. CD 14+ cells were purified using CD14 micro beads (Miltenyi). 1×10^6 cells/ml were cultured for 7 days in AIM-V media with 1× pen-strep (Gibco), 50ng/ml of hGM-CSF, and 50ng/ml of hIL-4 (Preprotech). mRNA was extracted from a neuroblastoma cell line (NGP) with the μMACS mRNA isolation kit (Miltenyi Biotech). DC were harvested and washed 2× with PBS. Cells were loaded with 2μg of NGP mRNA using DMIER according to the manufacturer's protocol (Invitrogen). After 4 hours, cells were washed 2× with PBS and phenotypic analysis of DC was performed by flow cytometry (Becton Dickinson FACSort). Cells were stained with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated antibodies against CD45, CD14, CD80, CD1a, CD83, CD86, DR, and CD11c for the expression of surface markers characteristic of immature DC. Intracellular staining was performed with GD2 (Chemicon) and CD56 antibodies after they were fixed and permeabilized. **Results:** The phenotype of DC (negative for CD14 and strongly positive for CD45, CD11c, and HLA-DR, dim positive for CD1a, CD80 and CD86 and negative for CD83) showing the immature state of these cells. Twelve experiments were done and in each experiment a median of 1×10^4 events were analyzed. A median of 2294 (range 493-7091) events was gated. Demonstrating positivity for intracellular GD2 in a median of 370 (range 210-2689) events. This represents a 30.4% (range 5.4-65.4%) transfection efficiency when compared to the positive control that demonstrated a median of 63.5% positive in 4181 events. **Conclusions:** We demonstrated the ability to transfect NGP mRNA into Immature Dendritic cells with a non-viral method achieving efficiencies of 30.4%. This step is one of many in the development of a DC vaccine against neuroblastoma.

220

ALTERED CD34 EXPRESSION OF UMBILICAL CORD BLOOD AFTER THAWING

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There is good correlation between pre-freezing umbilical cord blood (UCB) CD34+ cell dose and engraftment in pediatric transplant recipients. In our experience, when the cells from thawed and washed UCB units are analyzed there is up to a 2-fold lower recovery of CD34+ cells than one would predict based upon mononuclear cell recovery. This post-thaw CD34+ cell dose would appear insufficient to engraft patients consistently, yet does (data not shown). We hypothesize that CD34 expression is masked immediately after thawing and is restored in short-term culture. To test this, we analyzed the CD34 expression of cells in 5 thawed and washed UCB units at the time of thawing, and at 12, 24 and 48 hours afterwards. The pre-freezing CD34 expression data was not available as these UCB units were donated anonymously. The cells from each experiment were seeded at a concentration of 1×10^5 cells/ml in 3ml of the following culture media in a 37C, 5% CO2 humidified incubator: phosphate-buffered saline (PBS) with 10% bovine serum albumin (BSA) [control]; StemSpan (SS) liquid culture media (Stem Cell Technologies, Vancouver, BC, Canada) with 10% BSA; and SS with 10% BSA and a four-cytokine combination: thrombopoietin, Stem Cell Factor, Flt-3 ligand and Interleukin-3 (IL-3). The concentration of all cytokines was 50ng/ml except IL-3 (100ng/ml). At the set time points, a cell count was obtained and the cells were stained with the following monoclonal antibodies: CD45 FITC, CD34 ECD, and CD3 PC5. The stained cells were analyzed using a Beckman-Coulter FC500 cytometer. An ISHAGE gating strategy was implemented to eliminate cells with autofluorescence or nonspecific antibody binding. Viability (always $> 85\%$) and total cell counts did not significantly change during the time period studied in any of the culture conditions. The mean starting % of cells expressing CD34 was 0.03%. By hour 12, all cultured cells showed a 10-fold increase in CD34 expression ($p < 0.0005$, paired t-test); there were no significant differences amongst the different culture conditions and CD34 expression did not increase beyond 12 hours. Thus 12-hour culture of thawed UCB restored the proportion of CD34+ cells to within the reported range for fresh specimens (0.1-0.5%). This could explain, in part, why the engraftment characteristics of UCB units reflect their pre-freezing CD34 content rather than immediately after thawing. The exact mechanism of this shifting CD34 expression merits further investigation.

221

HEMATOPOIETIC STEM CELL (HSC) REDISTRIBUTION FOR CARDIAC REPAIR FOLLOWING ACUTE MYOCARDIAL INFARCTION (AMI)

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Controversy surrounds the role of HSC in repairing cardiac damage. In some animal studies, intracoronary or intramyocardial injection of autologous HSC contributed to new myocardial tissue following acute ischemic insult. The role of removing and reinjecting HSC is uncertain. HSC mobilized from the bone marrow circulate widely. We hypothesize that circulating HSC can localize to the area of revascularized cardiac damage following myocardial infarction and that HSC engraftment may have a beneficial effect on cardiac function. A pilot trial was conducted to study the feasibility and safety of HSC mobilization following AMI. Five patients that experienced large transmural anterior AMIs received 4 daily subcutaneous injections of 10 ug/kg G-CSF. At presentation, all patients had high-grade occlusions of a coronary artery that were successfully revascularized by percutaneous procedures. Baseline cardiac bloodflow (rubidium-82 PET) and myocardial metabolism (FDG-PET) studies confirmed transmural infarction. All patients started G-CSF within 7 days of AMI. Peripheral venous blood contained a median of 1.7 CD34(+)cells per ul (range 0.7 to 7.5) prior to G-CSF and a median of 75.6 CD34(+)cells per ul (range 72 to 91) following 4 days of G-CSF. There was no toxicity associated with G-CSF administration. The median baseline left ventricular ejection fraction (LVEj) was 30% (range 23-40%). Four weeks following HSC mobilization, the median LVEj increased to 40% (range 30 to 48%). This increase was clinically and statistically significant. Improvement in FDG uptake and a trend for increased perfusion were also observed. We conclude that administration of G-CSF following AMI is safe and mobilizes HSC into the circulation. G-CSF is associated with improved left ventricular function in a group of patients with large anterior wall AMI, a greater improvement than would be expected from historical controls. These findings suggest that G-CSF acts directly upon the heart to trigger recovery or G-CSF mobilized HSC migrate and engraft in the heart aiding recovery of cardiac function. A larger, randomized trial is planned to explore these issues.